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14. ABSTRACT

Our goal is to provide a more biologically relevant model of human physiology for the purpose of developing in vitro, cell based biosensors for environmental toxins. Specifically we plan to develop human neural based biosensors by using ArunA's neural cell lines derived from both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs).

This report describes the progress made in these major areas: (1) directed differentiation of hESC- and hiPSC-derived neural progenitor cells into dopaminergic neurons; (2) directed differentiation of hESC- and hiPSC-derived neural progenitor cells into astrocytes; (3) label-free, adhesive signature-based microfluidic cell separation; and (4) neural crest differentiation.

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425 River Road
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Quarterly Report
Human Neural Cell-Based Biosensor

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Summary

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This report describes the progress made in these major areas: (1) directed differentiation of hESC- and hiPSC-derived neural progenitor cells into dopaminergic neurons; (2) directed differentiation of hESC- and hiPSC-derived neural progenitor cells into astrocytes; (3) label-free, adhesive signature-based microfluidic cell separation; and (4) neural crest differentiation.

(1) Directed differentiation into dopaminergic neurons
We continue to make good progress on the development of a new dopaminergic progenitor cell line and dopaminergic differentiation kit for commercial release. We have narrowed down the best options for kit configurations and are continuing to optimize and edit a differentiation protocol for customer use. We are also currently in the process of repeating our dopamine release studies, as well as to further characterize our differentiated cultures for more extensive dopaminergic marker expression via immunocytochemistry and qPCR. We recently presented our results at the Annual Meeting of the Society for Neuroscience, October 13-17th, 2012, in New Orleans, LA. Preliminary work continues on translating dopaminergic neuron differentiation protocols to hiPSC-derived neural progenitor cells.

(2) Directed differentiation into astrocytes
We have made substantial progress on finishing studies delineating novel methods to differentiate hESC-derived neural progenitor cells into astrocytes significantly faster than current protocols in the literature. In fact, a manuscript on the astrocytic differentiation work has been submitted to *Stem Cell Research* for publication. Functional network based electrophysiological studies of astrocyte/neuron co-culture systems remain in progress. We are also continuing our work on translating our astrocytic differentiation protocols to hiPSC-derived neural progenitor cells.

(3) Label-free, adhesive signature-based microfluidic cell separation
Our collaboration with Georgia Tech is effectively underway toward developing a microfluidic-based approach to efficiently isolate different neural cell populations to shorten and streamline the scaled-up production of enriched populations of hiPSC-derived neural rosettes, neural progenitor cells and mature neuronal and glial cell types for direct use in cell-based assays. We are currently evaluating cell surface protein properties of our neural cell lines successfully generated under this contract in order to best optimize microfluidic cell separation methods.

(4) Neural crest differentiation
Separating and migrating from the neural tube during embryonic development and giving rise to a wide variety of cell types, neural crest cells have garnered increasing interest as new models of developmental neurotoxicity caused by environmental factors. Given the rising interest in neural crest cells as a means to create a human in vitro model to predict human health outcomes, we have begun preliminary work on differentiating our neural progenitor cells into neural crest cells following our own unique protocols.